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DECREASED DENSITY OF NEURONS IN THE MEDIAL PREOPTIC NUCLEUS AND INCREASED TESTICULAR WEIGHTS FOR RATS EXPOSED PERINATALLY TO AN 0.5 Hz ROTATING MAGNETIC FIELD

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Pregnant Wistar rats were exposed to either 0.5 Hz Rotating Magnetic Fields (50 microT to 300 microT or 1.5 mT to 3.0 mT) or to sham field or control environments for 3 days before to 3 days after the day of birth. The adult male offspring that had been exposed perinatally to the RMF displayed significantly heavier testicle weights but fewer neurons within the medial preoptic nucleus (MPO) and caudal ventromedial hypothalamus relative to sham field controls (effect size about 30%). There was no statistically significant difference between the two ranges of RMF intensities for these measures. RMF-exposed rats relative to sham-field exposed rats did not differ with respect to spleen weights, body weights, or mounting (reproductive) activity. The heavier testicle weights replicated previous studies and suggest that permanent, selective alterations in neuronal density can also occur subsequent to perinatal exposure to relatively weak, extremely low frequency magnetic fields.

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Changes in testicular weights of rats have been employed as indicators of perinatal sensitivity to extremely low frequency magnetic fields. Ossenkopp *et al.* (1972) reported that the testicular weights of adult

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male rats exposed either prenatally or perinatally to an 0.5 Hz Rotating Magnetic Field (RMF) were between 8% and 19% heavier than those of sham-field controls. More recently McGivern *et al.* (1990) found that the weights of accessory sex organs, including the epididymis, seminal vesicles and prostrate, were significantly heavier in adult rats exposed from prenatal days 15 through 20 for 15 min, twice per day to a 15 Hz magnetic field. The 18% increase in wet testicle weights only approached statistical significance. In both studies the intensities of the magnetic fields were in the order of about 1 mT (10 gauss).

Because: (1) permanent alterations in masculine sexual behavior can be affected by alterations of testosterone within the first few hours after birth (Roffi *et al.*, 1982), and (2) male-female differences are apparent within the medial preoptic nucleus of the hypothalamus within 24 hr after birth (Kuhnemann *et al.*, 1995; Madeira and Lieberman, 1995), we reasoned that: (1) neurons within this structure for the male rat may be particularly vulnerable to the biochemical correlates of perinatal magnetic field exposures, and (2) their differences should be detectable as changes in neuron density, and (3) these cell densities should be correlated significantly with testicle weights.

The following study was designed to discern if the earlier (Ossenkopp *et al.*, 1972) observations of increased testicular weights were replicable and if there were any significant correlations between the increased testicular size and neuronal densities within different regions of the ventromedial hypothalamus (VMH) and within the medial preoptic nucleus (MPO). These three structures were selected because of their sexual dimorphism (Madeira and Lieberman, 1995) and their strong correlations with the types of behaviours (Persinger, 1969; Persinger and Foster, 1970; Persinger and Pear, 1972; Persinger *et al.*, 1974) that have been measured following perinatal exposure to 0.5 Hz RMFs.

METHOD

Subjects

A total of 18, 90 to 130 day old Wistar albino female rats who had been impregnated 18 days previously (single night, verified by sperm

plugs), were exposed to one of four conditions: 1.5 mT to 3.0 mT RMF ($n = 6$), 50 microT to 300 microT RMF ($n = 6$), control (3 to 4 m distance) or sham-field ($n = 6$). The two exposure intensities were controlled by the spatial distance from the RMF. The sham-field exposure involved placing pregnant females ($n = 3$) within the same positions as the two RMF intensities except that the magnets had been removed. The control condition (3 litters) were maintained in a separate room within the colony.

Each pregnant rat was exposed singly in a 27 cm × 27 cm × 27 cm plastic, white cage (1.5 cm grid white plastic for the cover) from 3 days before to 3 days after birth to the experimental conditions (7 days). During this period food and water were available *ad libitum*. Three days after birth the litters were culled to 8 pups (4 males, 4 females). Temperature within the room was automatically controlled between 20 deg C and 21 deg C. The overhead white fluorescent lamps produced an intensity of 500 lux within the exposure regions. The light : dark ratio was 12 : 12 with the light onset at 0800 hr local time.

Exposure Procedure

The details of the RMF device have been reported previously (Persinger *et al.*, 1974). In summary, the RMF was generated by two horseshoe magnets rotating in opposite direction about their major axes at 0.5 Hz. The magnets were turned by a pulley-system driven by a Bodine motor. A gaussmeter showed a peak-to-peak (north polarity-to-south polarity) range of intensities between 1.5 mT to 3.0 mT within the region between the two magnets and between 50 microT to 300 microT at a perpendicular distance of 1 m. In this study the major axis around which the magnets turned was oriented southwest-northeast with the motor on the southwest side. Notation of these parameters may be relevant in light of the geometric relationship between the static field and time-varying field (Scaiano *et al.*, 1995).

Procedure

After the exposure period (postnatal day 3) to the experimental conditions the pups were placed with their mothers in standard plastic cages with 1/4 inch corncob as bedding. The pups were weaned at

21 days of age and designated to standard hanging wire cages (3 rats of the same sex/cage). When the male rats were 80 days male rats (at least one form each litter) were randomly selected and housed singly in a standard (for 3 rats) wire cage for about two weeks. For four successive nights, for two hours each, between 2300 hr and 0100 hrs (midscotophase) a female age-matched rat, in oestrus, was placed within the cage. Total numbers of mounts per night were recorded.

Within one week after the last observations of mounting the male rats ($n = 18$, 6 from each condition) were killed by decapitation. Wet weights for the two testicles and spleen (employed as a reference tissue) were obtained. The brains were removed and fixed in ethanol-acetic acid-formaldehyde before they were processed and embedded in paraffin (Persinger, 1988). Ten micrometer, coronal sections were obtained every 100 micrometers between the caudal and rostral boundaries of the hypothalamus. The sections were stained with toluidine blue 0. This method of fixation and staining yields sharp, clearly discriminable neuronal soma.

Sections from the caudal 1/3, middle, and rostral 1/3 of the VMH and medial preoptic nucleus were counted from the dorsal 1/3, middle and ventral 1/3 of the structure. This procedure was employed because of the potential differential neuronal input within these regions and the heterogeneity of receptor densities. Criteria for definition of the structures were obtained from Paxinos and Watson (1986). All regions were examined at 1000 X magnification with oil immersion. The neurons in each field were counted three times and the median value was used as datum. To be counted the neuron's nucleus was required to be visible. All slides were evaluated by the first author who did not know the condition to which the rat had been exposed. Data were analyzed by one way analysis of variance as a function of the two RMF-exposures and sham field/control rats and employed SPSS software. Post-hoc and confirmational analyses were also conducted. The probability value for statistical significance was set at $p < .01$.

RESULTS

The means and standard deviations for the total numbers of mounts (per 2 hours over 4 nights, *i.e.*, 8 hrs), wet testicular weights, wet spleen

weights, body weights, and numbers of neurons per unit area within the VMH and the medial preoptic nucleus of the hypothalamus for the rats that were exposed perinatally to the two RMF intensities or to the sham field/control setting are shown in Table I. The effect sizes (equivalent to the amount of variance in the dependent variable accommodated by the treatment, *i.e.*, eta-squared or the omega-squared estimate) are also presented.

One way analysis of variance (all $dfs = 2,17$) indicated that statistically significant group differences for testicle weights [$F = 5.83$, $p = .01$), the numbers of neurons within the caudal ventromedial hypothalamus [$F = 7.14$, $p < .01$], and the numbers of neurons within the medial preoptic nucleus [$F = 7.78$, $p < .01$]. *Post hoc* analyses (Tukey's, set $p < .05$) indicated that the source of the differences was the heavier testicle weights for the two RMF-exposed groups (that did not differ significantly from each other) relative to the sham/control-exposed rats. Similarly the source of the group differences for the neuronal densities was due to the significantly fewer neurons within the caudal VMH and the preoptic region for both of the RMF-exposed groups relative to the reference group. There were no statistically significant group differences (all $ps > .05$) for spleen weights, body weights, numbers of mounts during sexual activity or numbers of neurons within the larger area of the VMH.

We applied a trend analysis (polynomial) to discern if the relationship between the measurements and the intensity of the exposure was linear for testicular weights and the statistically significant neuronal counts. The relationships (all $dfs = 2,14$) between the RMF intensity and the testicle weights [$F = 9.08$, $p < .01$], numbers of neurons in the caudal VMH [$F = 12.75$, $p < .01$] and medial preoptic region [$F = 16.56$, $p < .001$] were linear and did not deviate from significantly from a linear relationship.

Both Spearman rho (nonparametric) and Pearson r (parametric) correlations (verified by scattergrams) were comparable in magnitude and significance and showed statistically significant ($p < .01$) associations between the density of neurons within the MPO and testicle size ($rho = -.68$), spleen size ($rho = -.70$) and body weight ($rho = -0.64$) but not mounting behavior ($rho = -.02$). Only the neuronal density within the dorsal caudal VMH was associated with testicle weight ($rho = -.63$).

TABLE I Testicular weights (gm), spleen weights (gm), body weights (gm), numbers of mounts (total of 8 hr) and the numbers of neurons per 1 mm-sq within the medial preoptic nucleus (MPO), and ventromedial nucleus (VM) of the hypothalamus in male rats exposed perinatally to sham-fields or to Rotating Magnetic Fields

			RMF-intensity			
	Sham-controls		*Low*		*High*	
	6		6		6	
Variable n	*M*	*SD*	*M*	*SD*	*M*	*SD*
Tissue						
testicles (g)	3.64	.34	4.00*	.26	4.04*	.11
spleen (g)	1.02	.19	1.02	.24	1.19	.18
General						
body weight (g)	467	30	474	52	505	35
mounts (number)	107	25	115	49	68	30
Hypothalamic Nuclei						
(Numbers/mm-squared)						
Ventromedial (VMH)	455	58	399	36	405	23
VMH (caudal shell)	560	73	424*	62	387*	95
Medial Preoptic N.	529	42	439*	80	367*	77

*$p < .05$ from control/sham group.

DISCUSSION

The results of this study replicated previous experiments (Ossenkopp *et al.*, 1972; McGivern *et al.*, 1990) that perinatal exposure to extremely low frequency magnetic fields can be associated with increased wet weights of testicles. About one-third of the variance in the testicle weights was accommodated by the treatments; the magnitude of the effect size and the direction and the proportional increase (about 10%) were similar to that of the study 25 years ago (Ossenkopp *et al.*, 1972). The observation that there were no statistically significant differences between the body weights and spleen weights for the treatments suggests that the testicular effect may be specific to the field exposures.

The additional contribution of the present study was the measurement that the rats exposed perinatally to the RMF exhibited significantly fewer neurons within the medial preoptic nucleus (MPO) of the hypothalamus compared to the non-exposed brains. The effect size was comparable to that of the testicular differences. That the 20% reduction in neurons within the MPO of rats exposed perinatally to the RMF was not a nonspecific effect was suggested by the absence of group differences for the numbers of neurons averaged within the larger area of the VMH. In addition, a review of the widths and lengths of cerebrums (Tab. II) from an unpublished doctoral dissertation (Persinger, 1971) that employed the same procedures and apparatus as the present study indicated that these exposure parameters and intensities did not significantly affect the gross size of the brains of the rats although their testicular weights were affected (Ossenkopp *et al.*, 1972).

TABLE II Means and standard deviations for rostral-caudal cerebral lengths (in mm) and maximum widths (in mm) for a different group of rats exposed perinatally to the same RMF intensities or sham-field conditions

	Length			*Width*		
		RMF			*RMF*	
	Sham	*Low*	*High*	*Sham*	*Low*	*High*
N	8	8	8	8	8	8
M	16.6	16.6	16.1	15.6	15.7	15.9
SD	.5	.4	1.0	.7	1.0	.9

The results of this study would also be consistent with the hypothesis of McGivern *et al.* (1990) that low frequency magnetic fields, either time-varying or evoked by amplitude modulation of radiofrequency (MHz to GHz range) carriers, activates ornithine decarboxylase (ODC) or induces long-term increase in the enzymatic conversion of testosterone to dihydrotestosterone. The latter regulates prostatic growth and differentiation. Because ODC is essential for growth in all eukaryotic cells, appropriate magnetic field stimulation would be expected to induce general as well as organ-specific effects.

There is additional empirical support for the McGivern *et al.* (1990) hypothesis concerning the role of enzymes involved with the urea cycle. We (Persinger *et al.*, 1978) found a 20% reduction in the amount of blood urea nitrogen (BUN) and aspartate aminotransferase activity in adult rats that had been exposed perinatally to the RMF (Persinger *et al.*, 1978). About 20 other measures of blood electrolytes and enzyme activity were not affected significantly. If one assumed that changes in the donation of nitrogen from aspartate to citrulline was reflected by the decreased aspartate aminotransferase activity and the alterations in the release of urea during conversion from arginine to ornithine could be inferred by the decreased BUN, then these two specific changes would be consistent with involvement of the ornithine pathway.

The contribution of ODC to the reduction of neurons within the MPO and the caudal shell of the VMH, although speculative, would be consistent with previous data. If we assume that: (1) the normal enhancement of oestrogen receptors occurred within the MPO (Kuhnemann *et al.*, 1995), (2) an RMF-induced enhancement of the conversion of elevated testosterone (Roffi *et al.*, 1987) to dihydrotestosterone by ornithine decarboxylase also encouraged the further aromatization to oestradiol (McEwen, 1981), (3) this elevated level of oestradiol helped maintain oestrogen receptor density, and (4) this type of maintained density of oestrogen receptors is associated with the type of reduction in neuronal numbers that has been reported for the cerebral cortices (Diamond, 1988) then the present experimental results might be explained. It is interesting that the differential between male and female rats for postnatal oestrogen receptors and the numbers of neurons within the cerebral cortices is about 20%.

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